

Docket No. MWH-0029US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Stephen B. Liggett
Application No.: 09/856,803
Filed: May 25, 2001 (35 U.S.C. § 371 of PCT/US99/27963, filed November 24, 1999, which claims benefit of U.S. Appl. No. 60/109,886, filed November 25, 1998)
Confirmation No.: 3706
Group No.: 1634
Examiner: Myers, C.
For: POLYMORPHISMS IN THE 5' LEADER CISTRON OF THE β_2 -ADRENERGIC RECEPTOR

Commissioner for Patents
Washington, D.C. 20231

Certificate of Facsimile Transmission
I hereby certify under 37 C.F.R. § 1.8 that this correspondence is being transmitted by facsimile to the United States Patent and Trademark Office, Commissioner for Patents, TC 1600, at (703) 872-9306, on 10-12-03.


Matthew M. Carlett

DECLARATION OF STEPHEN B. LIGGETT, M.D., UNDER 37 C.F.R. § 1.131

This Declaration Of Stephen B. Liggett, M.D., Under 37 C.F.R. § 1.131 is being submitted as part of Applicant's Response To Office Action Under 37 C.F.R. § 1.11 regarding the office action dated January 7, 2003 that was received in the captioned application.

Being warned that willful false statements and the like are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements and the like may jeopardize the validity of the instant application or patent resulting therefrom, I hereby declare that:

1) I am the original, sole, and first inventor of the subject matter that is claimed in pending claims 1-8 and 11 of the captioned application, namely (a) a method for genotyping the

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β_2 -adrenergic receptor (β_2 AR) gene of an individual comprising determining the identity of the nucleotide pair at the 5' leader cistron (5'LC) polymorphic site (PS), which, as is demonstrated throughout the specification of the captioned application, is located 47 bases upstream of the β_2 AR coding region, which begins at nucleotide position 1588 of SEQ ID NO:1 (thus, the 5'LC PS is located at nucleotide position 1541 of SEQ ID NO:1) in the two copies of the β_2 AR gene present in the individual; and (b) a method for genotyping the β_2 AR gene of an individual comprising determining the identity of the nucleotide pair at the 5'LC PS and at one or more additional PSs in the β_2 AR gene in the two copies of the β_2 AR gene present in the individual.

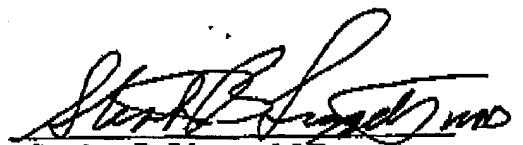
2) Further to an effort, dating back to as early as January of 1996 (see attached copies of PCR protocols), to discover polymorphisms in the region upstream of the β_2 AR gene, I directed the performance of an experiment designed to elucidate the existence, if any, of such polymorphisms. Utilizing PCR techniques to analyze genomic DNA in this region from human volunteers, I discovered, in the "sense" strand, the existence of a thymine residue 47 bases upstream of the β_2 AR coding region, as well as the existence of an adenine residue 47 bases upstream of the β_2 AR coding region in the "antisense" strand. Copies of chromatograms generated by the automated sequencer used to sequence the PCR products demonstrating this discovery are attached (chromatogram #096-1369 demonstrates a thymine residue in the "sense" strand at the nucleotide position that is located 47 bases upstream of the β_2 AR coding region; chromatogram #096-1364 demonstrates an adenine residue in the "antisense" strand at the nucleotide position that is located 47 bases upstream of the β_2 AR coding region; chromatogram #096-1367 demonstrates a thymine residue in the "sense" strand at the nucleotide position that is located 47 bases upstream of the β_2 AR coding region; and chromatogram #096-1362 demonstrates an adenine residue in the "antisense" strand at the nucleotide position that is located 47 bases upstream of the β_2 AR coding region). Although all previous reports indicated that the only known residue at the nucleotide position located 47 bases upstream of the β_2 AR

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coding region, in the "sense" strand, was a cytosine (and thus, in the "antisense" strand, a guanine), to confirm that I had indeed discovered a polymorphism at this position. I subsequently directed the performance of a similar experiment with the wild-type sequence, and discovered, in the "sense" strand, a cytosine, and in the "antisense" strand, a guanine. Copies of chromatograms generated by the automated sequencer used to sequence the PCR products demonstrating this discovery are attached (chromatogram #096-2859 demonstrates a cytosine residue in the "sense" strand at the nucleotide position that is located 47 bases upstream of the β_2 AR coding region; and chromatogram #096-2860 demonstrates a guanine residue in the "antisense" strand at the nucleotide position that is located 47 bases upstream of the β_2 AR coding region). My discovery of this polymorphism, and my subsequent confirmation of this discovery, occurred prior to the effective date of any of the following references: Timmermann *et al.*, *Kidney Intl.* 53:1455-60 (June 1998), Timmerman *et al.*, *J. Molecular Med.* 76:B30, Abst. P-109 (May 1998), Timmermann *et al.*, *Human Mutation* 11(4):343-4 (March 1998). With respect to the copies of the chromatograms, the nucleotide position that is 47 bases upstream of the β_2 AR coding region is that denoted with a "^" symbol.

3) All statements made herein of my knowledge are true, and all statements made herein on information and belief are believed by me to be true.



Stephen B. Liggett, M.D.
Director, Division of Pulmonary and
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University of Cincinnati Medical Center

OCT. 13. 2003 9:48AM

GENAISSANCE PHARM.

NO. 191 P. 19



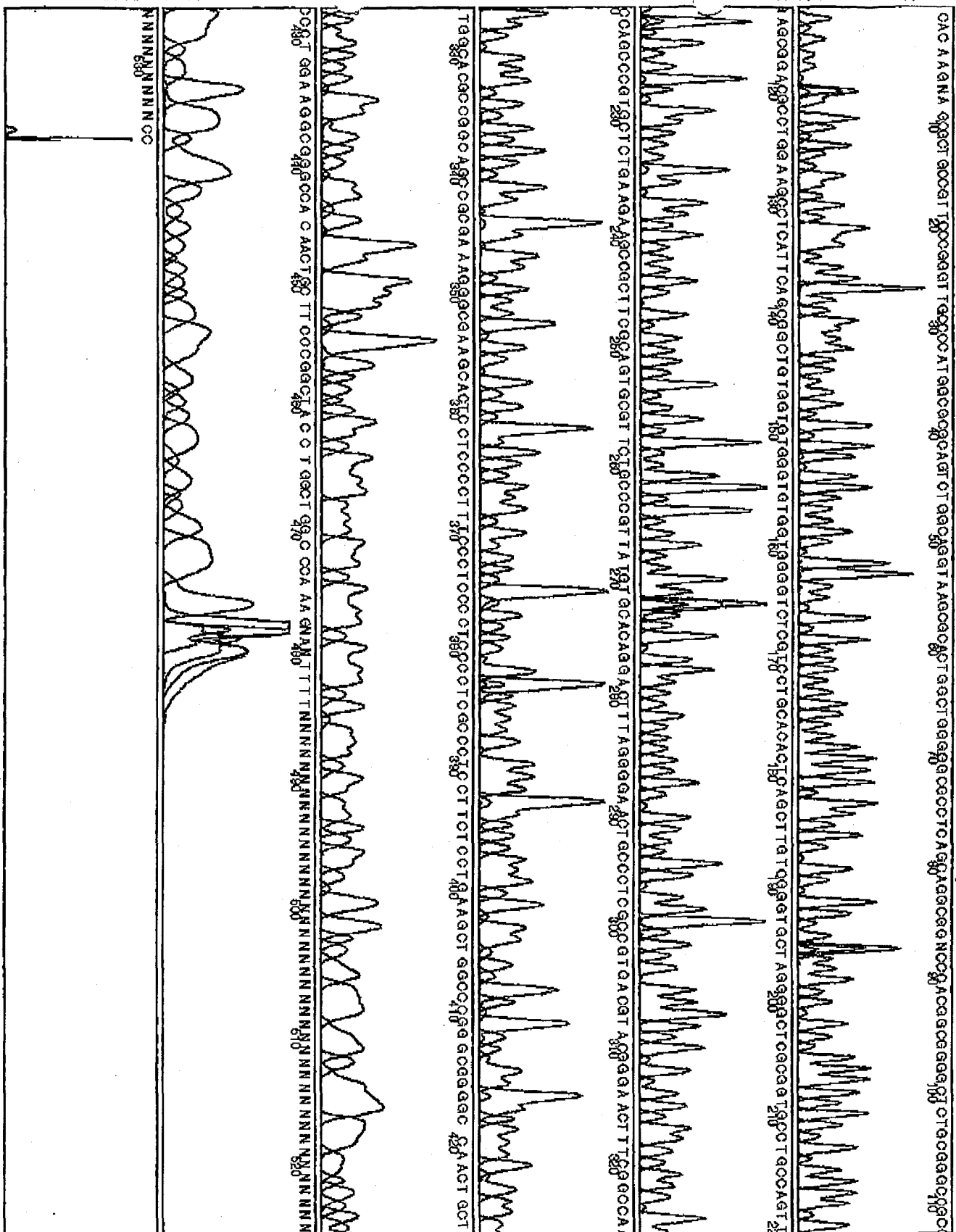
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Version 2.0.1S

B3/9 16
Dye Terminator (Any Primer)
Lane 16
Signal: G:1186 A:1244 T:584 C:560

INST FILE 808296
096-1364
MCGRAW-A-2R-1

Tue, Mar 5, 1996 8:51 PM
X: 0 to 6640 Y: 0 to 1600
Spacing: 10.38

A2-R





Model 373A
Version 2.0.15

B3/S 18
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 Lane 18
 Signal: G:1128 A:1741 T:517 C:580

INST FILE 808296
098-1387
MOGRAW-DWMF-1

Tue, Mar 5, 1996 8:51 PM
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Page 1 of 1
Alma-F

CNCNCTC T 09JGECCECCMAJGGT AGCCCGEGG AAGCAAT GGT JGGCCCCCCCOT JCACAGENMAGJAJT TGAGGCCCGJGCOC CAGABOO AJCCOTCAAG ANA AGGASAGGCGJ NAGAG NAGAG TJ GGG

AAAGGCGNAGNATGGCTCACCCTT₁₈₀GACACTACCGGCTACCAT₁₈₀TGCCGAAGA₇₀TCCCATACGTCAAGGCGAAGGCACAGTCAGTTCCCTAAATCTCT₂₁₀QACACATA₂₂₀

CAGAAGGACCTGCGAAGCCAGCTTCTTGGAGAGGACGAGCTGGAAGCTGGCAGGCGACCGGAGGCCCTATTCACCCGAGACAAGGTGAGTGTGGAGGAGGAAATCCGACACACAC

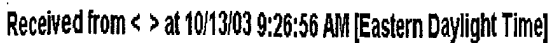
380 CACACCGA GCGCGCTGA 380 GAAGCTTCGANGCCT CCGGTCGCGGCC 385
390 CAAACGCCCGCCATGAGT 400 GCGCTGCTGAGC 410
A 420 GCGCAATGCGCT 430

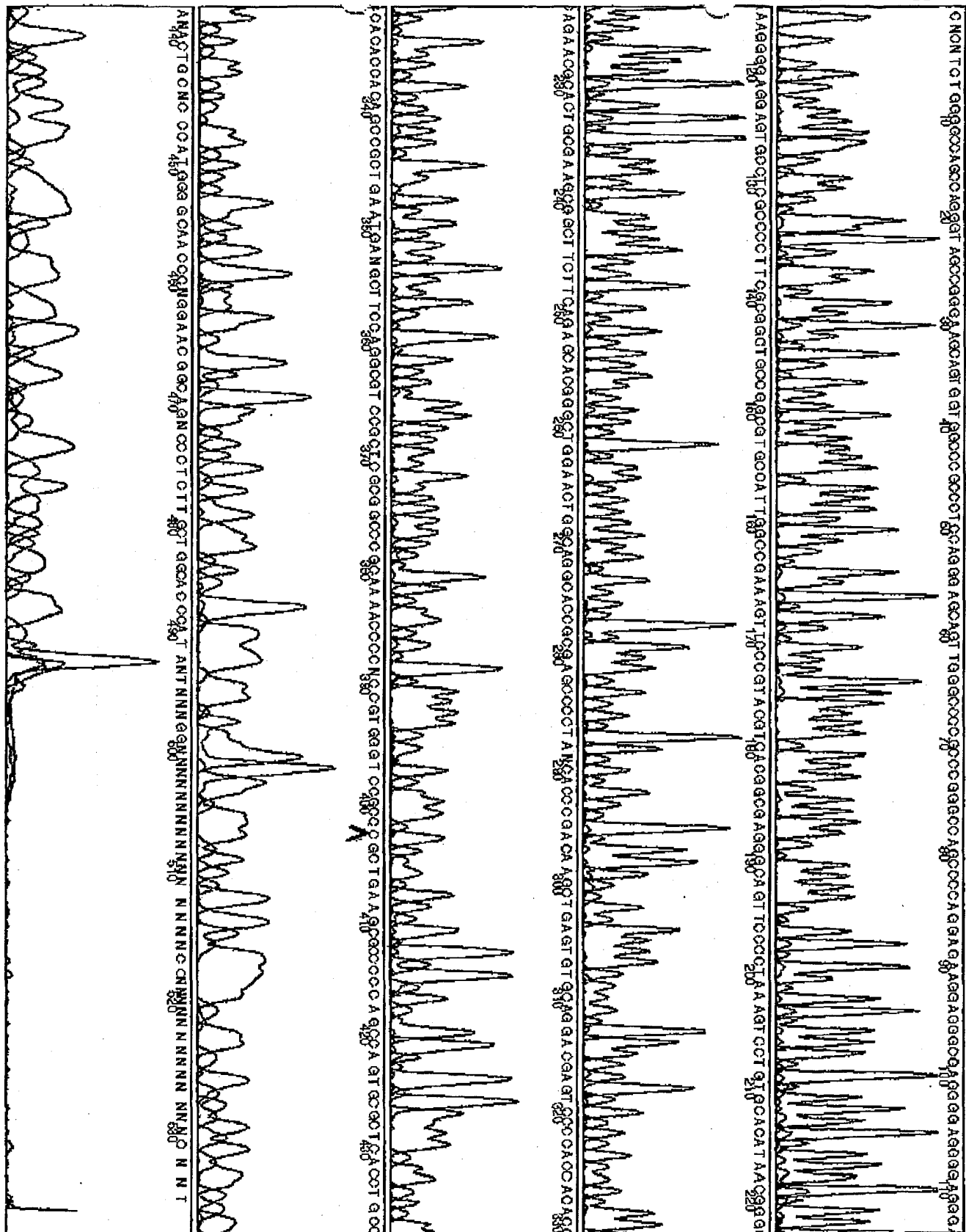
[illegible]

830
NNNNNNCO

DUM-R

P. 21





Received from < > at 10/13/03 9:26:56 AM [Eastern Daylight Time]

PCR (BAR)

1/26/96

* Made new set of primers for BAR 5' flanking region. ~~Both~~
Product should include the CRE + short ORF. Primers
were chosen in MacVector

Blood samples are being obtained from pts in the UC asthma
clinic by Melanie Meyer.

DNA samples prepared by Liz Donnelly using Amplitaq & 10
Dianz kits. Samples are numbered as received (A1, A2, etc.)

- Set up PCR rxn using new primers. Made up 96ul master mix.

10ul buffer II

6ul 25mM MgCl₂

0.8ul 25mM dNTP

0.5ul 100uM forward primer (BAR-F1)

0.5ul 100uM reverse primer (BAR-R1)

77.7ul dH₂O

0.5ul ampliq

- aliquot 24ul of master mix into PCR tube

- add 1ul template DNA

- overlay with 1 drop mineral oil

- perform PCR in thermal cycler with denaturation made on

94°C x 2 min

94°C x 30 sec / 64°C, 62°C, 60°C, or 58°C x 30 sec / 72°C x 30 sec => 35 cycles

72°C x 7 min

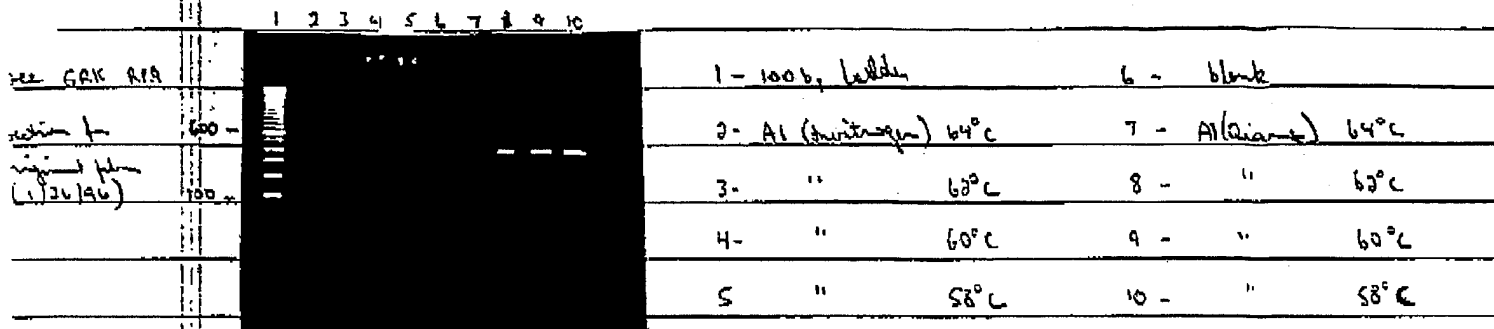
- Run 10ul of PCR rxn on TBE minigel

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NO. 191 P. 25

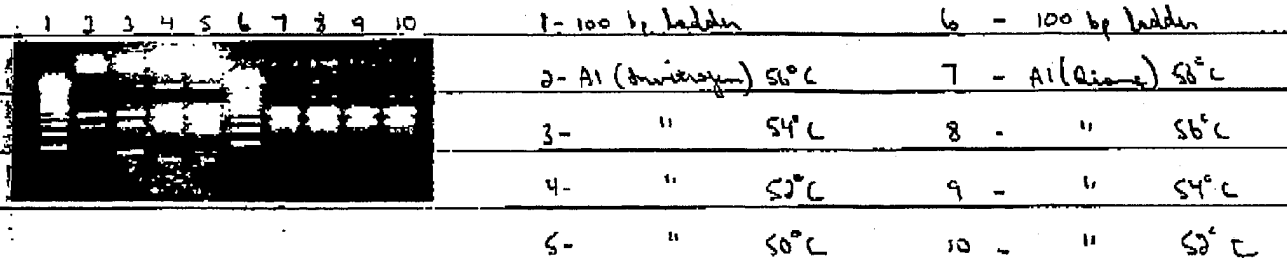
1/26/96 (cont)



No product seen = DNA isolated by Amirgen kit. Nice band
seen = DNA from Qiana kit but expected size should be
528 bp. The band present appears to be < 400 bp.

Repeat PCR runs = following modifications:

- add 73.7 ul dH₂O
- digest 23 ul of master mix into PCR tubes
- add 2 ul template DNA
- change cycling temp for Amirgen template to 52°, 54°, 53°, 50°C ; change
cycling temp for Qiana template to 58°, 56°, 54°, 53°C.



Now have bands in all samples. However, apparent band
present in both samples sets appears to be < 500 bp, still
smaller than expected.

B₂AR PCR (5th run)

Set up master mix for four (4) 25 ul PCR rxns:

2. ul	template DNA (DWM)
1.5 ul	forward primer
1.5 ul	reverse primer
20 ul	SX buffer (buffer N from Phastage PCR optimization kit)
10 ul	dNTPs (2.5mM) (from optimization kit)
65 ul	dH ₂ O
0.8 ul	Tag
100 ul	total

- aliquot 25 ul of master mix into 4 PCR rxn tubes
- overlay w/ 1 drop of mineral oil
- PCR cycle

98°C	2 min	} 30 cycles
98°C	30 sec	
56° 54° 52° or 50°	30 sec	
72°C	30 sec	
72°C	7 min	
- remove 10 ul aliquot & run on minigel

- #1 - 100 bp ladder
- #2 - 56°C
- #3 - 54°C
- #4 - ~~56~~ 52°C
- #5 - 50°C

